REMARKS

Claims 1-17, 46 and 54 are pending in the application. Withdrawn claims 12, 18, 32, 50, and 54 have been canceled, without prejudice to their inclusion in one or more related applications, in view of the final Restriction Requirement.

Paragraphs [0010], [0013] and [0018] of the specification have been amended to correct insert inadvertently omitted terms in paragraphs [0010] and [0018], to correct punctuation in paragraph [0010], and to correct a grammatical error in paragraph [0013]. In paragraph [0010], prior to the correction, the sentence referring to the porous barrier function did not make sense in view of the inadvertent omission of the term "prevents." The inclusion of the term in the clause "a barrier that prevents intermixing of stem and mammalian cells" now makes sense and is consistent with the same concept at page 41, line 25 ("the two cell types are separated by a porous barrier...."). The other amendments to paragraph [0010] are to correct punctuation by substituting a comma for an opening parenthesis. Accordingly, no new matter has been added to amended paragraph [0010].

Paragraph [0013] has been amended to refer to the singular term "disorder", rather than the plural term "disorders." No new matter has been added to amended paragraph [0013].

In paragraph [0018], prior to the correction, the sentence referring to the nucleic acids did not make sense in view of the inadvertent omission of the term "nucleic acid." The inclusion of the term in the clause "The nucleic acid can be selected from the group consisting of a nucleic acid encoding..." now makes sense and is consistent with the rest of the paragraph. Again, no new matter has been added.

Entry of the amendments to the specification is respectfully solicited.

Claim 4 has been amended to indicate that the size of the pores of the porous membrane are such that molecules having a molecular weight of less than 50,000 can pass through the pores. This amendment is supported at page 14, line 27, of the application as filed.

Claim 5 has been amended to incorporate from original claim 10 the reagent being an antibody. Claim 10, which would have been redundant in view of this amendment, has been canceled.

Claim 17 has been amended to focus on the differentiated human cell being a skeletal muscle cell, an endothelial cell or a differentiated hematopoietic cell. all as supported clearly by the results of Examples 2-5.

No new matter has been added by any of the amendments, which are fully supported by the application as filed. Accordingly, entry of the amendments to the claims is respectfully solicited.

Claims 1, 11, 13-17, and 46 stand rejected.

Rejection Under the First Paragraph of 35 U.S.C. § 112.

The Examiner has rejected claims 1-11, 13-17, and 46 under 35 U.S.C. § 112, first paragraph, asserting that the claims contain subject matter that was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention. Applicant traverses each of these grounds of rejection.

Specifically, the Examiner has alleged lack of enablement on four grounds:

(1) The specification allegedly does not make clear "how homogenous" the population of natal $CD34^+KDR^+$primitive stem cells that give rise to both hematopietic and stromal cell populations is;

(2) it is allegedly "not clear" from the specification how the injected human donor post natal $CD34^+KDR^+$ cells differentiated into any specific cell type as claimed in claim 17, as there is allegedly a lack of characterization of these cells by phenotype or functional capacity;

(3) the specification allegedly does not teach how to extrapolate data obtained from the *in vivo* studies of Examples 4 and 5, wherein post natal $CD34^+KDR^+$ cells were injected in non-immuno-compromised murine blastocytes or injected into the regenerating murine muscle to the development of effective *in vivo* or *in vitro* methods of generating a differentiating human cell that specifics selected type; and

(4) the specification allegedly does not reasonably provide enablement for a method of generating a differentiating human cell type of a selected type wherein the stem cell is separated from the differentiating mammalian cell by *any* porous barrier as previously claimed in claim 4.

In view of the positions noted above, the Examiner concluded:

> In view of the quantity of experimentation necessary, the unpredictability of the art, the lack of sufficient guidance in the specification, the limited working examples, and the limited amount of direction provided given the

> breadth of the claims, it would take undue trials and errors [*sic*] to practice the claimed invention.

More specifically, with respect to point 1, the Examiner asserted the following:

> [I]t is not clear from the specification how homogeneous is the population of natal CD34+KDR+ primitive stem cell that give rise to both hematopoietic and tromal stem cell population. There is no disclososure [*sic*] on how homologeneous is this cell population (e.g. 90%, 95% or 99%). It is very possible that the cell population contain heterogeneous cell population that give rise to both hemopoietic and stromal elements. What was the sensitivity of the method for selecting natal CD34+KDR+ primitive stem cell, what is the accurate and reproducible quantification of such selection. One skilled in the art would not know the homogeneous nature of the natal CD34+KDR+ primitive stem cell using the teaching of the specification alone.

The Examiner relied upon Waller et al. (Blood, 1995, **85**, pp2422-2435) (hereinafter "Waller"), for the proposition that there is no evidence that a single cell can differentiate along both a hematopoietic and stromal lineage (citing page 2434), and also relied upon Holden et al. (Science, 2002, **296**, pp 2126-2129) (hereinafter "Holden") for the proposition that there is no evidence that purified blood stem cells can contribute to any other tissue (citing page 2127).

With respect to point (1), the Examiner's attention is directed to paragraph [0069] at page discloses that starvation-resistant cells isolated according to the present invention are essentially 100% KDR^+.

Moreover, paragraph [0112] at pages 38-39 of the application states the following with respect to the purity of the isolated KDR^+ cells:

> "Isolated" refers to a cell that has been selected from other cells based on a specific characteristic (e.g., expression of a cell surface marker, cell shape, cell size, and the like). Likewise, "isolating" refers to the process of selecting a cell from other cells based on a specific characteristic. For example, an "isolated KDR^+ cell" is a cell that has been selected out of a population or group of other cells on the basis of KDR expression. A population of "isolated cells" is comprised of a group of similar cells that have been selected from a larger, heterogeneous group of cells based on a specific characteristic. For example, a population of isolated stem cells is a group of cells that has been selected from a larger group of cells comprising stem cells and non-stem cells, based on stem cell characteristics, such as those described herein. Isolated KDR^+ cells are preferably present in a population that is at least about 80% KDR+ cells, and more preferably at least 90% KDR^+ cells. Typically, a population of

> cells referred to herein as isolated KDR^+ cells means a population in which about 80% to 100% of the cells express the KDR marker. Although cell populations comprising fewer than 80% KDR^+ cells can be used in the methods described herein, the efficiency of the methods will generally decrease as the proportion of KDR^+ cells in the population decreases.

Based on these disclosures, one skilled in the art would readily understand that the population of isolated KDR^+ cells should be as homogenous as possible. The specification also discloses how to achieve such highly homogenous population by the isolation techniques disclosed in paragraphs [0016] and [0028], for example.

Furthermore, Figs. 1E and 1F of the present application show purity of at least 98% $CD34^+$ cell populations. See the description of these Figs. at paragraph [0032] at page 9 and paragraph [0169] at page 49.

Applicant respectfully submits that the findings reported in Waller are not particularly applicable to the present application, as the cell population studied in Waller is a bone marrow-derived $CD34^+$,$CD38^-$ cell population, and not a $CD34^+KDR^+$ cell population. These are distinct cell populations. See, for example, Ziegler et al., Science, 1999, **285**, 1553-1558, (hereinafter "Ziegler 1999") a copy of which was submitted with the Request for Reconsideration filed January 29, 2004, at Fig. 1B, which shows that a portion of $CD34^+KDR^+$ cells is 38^- and a portion is 38^+ and that about 1% of $CD34^+$ cells are KDR+ and about 5-10% of $CD34^+$ cells are 38^-. The Ziegler 1999 Figs. 1A and 1B are reproduced in the present application as Figs. 1A-1F and 1G-1 through 1G-14, respectively, and also in the parent application, now U.S. Patent 6,586,192 B1 (hereinafter the "Parent Patent").

With respect to the Examiner's citation of Waller for the proposition that there is no evidence that a single cell can differentiate along both a hematopoietic and stromal lineage, Applicant respectively disagrees. Example 2 at page 62 of the present application contains such evidence that $CD34^+KDR^+$ cells resulted in both hematopoietic and endothelial cell precursors.

More specifically with respect to point (2), relating to the diverse cell types of former claim 17, the Examiner stated:

> There is no characterization of these cells as to phenotype or functional capacity. It is possible that these cells are an irrelevant contamination of the stem cells selection process or do not provide function associated with stromal microenvironment. Moreover, there is no evidence from the Specification that there was no fusion of the $CD34^+KDR^+$ cells with cells

> of the other lineages. Holden et al. (Science, 2002, V.296, pages 2126-2129) teach that cells can mutate and develop markers characteristics of other lineages or that cells injected into a foreign tissue can take up local DNA and thus appears to have changes identify (see page 2126 in particular). Moreover, Holden et al. further teach that fusion scare has given further impetus to effort to establish rigorous standards for demonstrating plasticity such as: the cells must be properly identified at the outset, because a single alien cell in ostensible purified culture could produce misleading results. The cells must contribute to the function of the host tissue. There is no indication that demonstrate functionality of said cells in the specification. [original emphasis]

The Holden article is generally directed to and discusses some perceived flaws in previous research upon which conclusions of cell plasticity have been drawn. The Examiner seems to focus on the comments of Diane Krause of Yale University. Holden reports that Krause now questions her own work which apparently demonstrated that mast blood stem cells implanted into mice had progeny that were incorporated into lung, skin, intestine and liver cells. According to Holden, she and other researchers now consider that her result may have been an artifact of a cell fusion event that she did not control for or detect.

Regardless of the mechanism, Example 2 shows in vitro evidence that the $CD34^+KDR^+$ cells resulted in both hematopoietic and endothelial cell precursors. Likewise, Example 3 at pages 63 – 64 of the application detected hemoangioblasts resulting from $CD34^+KDR^+$ cells, where the hemoangioblasts tested positive for both hematopoietic and endothelial cell markers. Further, Example 4 at pages 64 – 65 of the present application, $CD34^+KDR^+$ cells injected into murine blastocysts resulted in newborn mice having human/mouse chimerism in multiple tissues, including tissue of the central nervous system, (brain and spinal cord) and tissues of endodermic or mesodermic origin (*e.g.* liver, lung, gut, skeletal muscle, heart and kidney). Still further, Example 5 at pages 65 ad 66 of the present application showed that $CD34^+KDR^+$ cells or $CD34^-lin^-KDR^+$ cells from peripheral blood and cord blood differentiated into mesenchymal tissues other than the original tissue, and specifically skeletal muscle cells. These examples provide further evidence that the $CD34^+KDR^+$ cells differentiate into other tissues. Patent law does not require an accurate explanation of the theory or mechanism for the functional results as demonstrated in the present application.

Further with respect to point (2), the Examiner appears to be questioning whether the differentiation recited in the claim actually occurs, contending that there is no characterization of

the resultant cells as to phenotype or functional capacity. Applicant respectfully disagrees as just explained with respect to Examples 2-5.

The Examiner's attention is also directed to the following recently published journal articles which corroborate Applicant's conclusion about the differentiation of the $CD34^+KDR^+$ cells into skeletal muscle cells, hematopoietic cells and endothelial cells. Applicant is the corresponding author in each and copies are attached to this Amendment: R. Botta et al., The FASEB Journal, Heart infarct in NOD-SCID mice: Therapeutic vasculogenesis by transplantation of human CD34+ cells and low dosed CD34+KDR+ cells, express article 10.1096/fj.03-0879fje, published online July 1, 2004 (disclosing differentiation of the $CD34^+KDR^+$ cells into hematopoietic cells and endothelial cells); E. Pelosi et al., 2002, Blood, Identification of the Hemangioblast in postnatal life, **100**, pp. 3203-3208 (also disclosing differentiation of the $CD34^+KDR^+$ cells into hematopoietic cells and endothelial cells); and P. Madeddu, et al., The FASEB Journal, Transplantation of low dose $CD34^+Kdr^+$ cells promotes vascular and muscular regeneration in ischemic limbs, express article 10.1096/fj.04-2192fje, published online September 2, 2004 (disclosing differentiation of the $CD34^+KDR^+$ cells into endothelial cells and apparently skeletal muscle cells).

Claim 17 has been amended to focus on the differentiation of the $CD34^+KDR^+$ cells into skeletal muscle cells, hematopoietic cells and endothelial cells, all as specifically supported by the data of Examples 2-5. Accordingly, the Examiner's concern about the ability of the $CD34^+KDR^+$ cells to differentiate into any of the cells previously recited in claim 17 should now be overcome. Reconsideration and withdrawal of the rejection at least of claim 17 are respectfully requested.

The Examiner contended that a person of skill in the art would not have understood the applicability of the method *in vitro* to an *in vivo* context (point (3), above). Applicant again respectfully disagrees. The examples themselves and the extensive citation of references in the application relating to the techniques involved would be more than adequate to enable a person having a high level of skill in this art, based on the description in the specification would have understood how to make and use the invention both *in vitro* and *in vivo.*

Regarding point (4), the Examiner contended that the specification does not enable claim 4 where the cell is separated by just any porous membrane. In view of the amendment to claim 4 made herein relating to the size of the pores being such that they allow molecules of less than

50,000 MW to pass through the porous membrane, it is submitted that the Examiner's rejection no longer applies. Reconsideration and withdrawal of the rejection of claim 4 are respectfully requested.

In view of the foregoing discussion, it is respectfully submitted that the Examiner's grounds of rejection based upon 35 U.S.C. § 112, first paragraph, for lack of enablement, have been overcome and/or are no longer applicable. Reconsideration and withdrawal of the rejection with respect to all claims are respectfully requested.

Rejection Under First Paragraph 35 U.S.C. § 112 - Written Description.

The Examiner has rejected claims 1-11, 13-17, and claim 46 under 35 U.S.C. § 112, first paragraph, asserting that the claims contain subject matter that is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor at the time the application was filed had possession of the invention. Specifically, the Examiner contended that the specification fails to describe any reagent other than an antibody that specifically binds with KDR. Therefore, claims reciting the reagent lack support of the written description. In view of the amendment to claim 5, incorporating an antibody that specifically binds with KDR as the reagent from original, but now canceled claim 10, Applicant respectfully submits that this rejection has been overcome with respect to claims 5-9. These were the only claims directly or indirectly reciting a reagent.

Applicant respectfully traverses the rejection as it applies to claims 1-4, 13, 14 and 46. Claims 1-4, 13, 14 and 46 do not recite use of a reagent that specifically binds with KDR in order to isolate a stem cell from human hematopoietic tissue. The only claims that recited a "reagent" element were claims 5-10. Since the amendment to claim 5 has overcome this rejection with respect to claims 5-9, and the rejection is not applicable to claims 1-4, 13, 14 and 46, withdrawal of the rejection is requested with respect to all of the claims.

Rejection Under 35 U.S.C. § 103(a).

The Examiner has rejected claims 1-3, 5-10, 13-17, and 46 under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,736,396 of Bruder, *et al.* (hereinafter "Bruder"), taken in view of U.S. Patent No. 5,912,133 of Lemischka (hereinafter "Lemischka"), and in view of the present specification disclosure at page 63, line 48, and page 4, lines 4-10. In particular, the

Examiner asserted that Bruder teaches a method of generating a differentiated cell of a selected type by incubation of human mesechymal stem cells in the presence of differentiating mammalian cells or a "conditioned medium" that are effective to induce the differentiation into a lineage of choice. The Examiner conceded that Bruder does not teach that the stem cells are human KDR^+ stem cells. Lemischka, according to the Examiner, teaches a method of isolating human FLK^+ stem cells using an antibody that specifically binds FLK-1. The Examiner characterized the cited portions of the present specification as teaching that human KDR^+ cells are the "same subpopulation" of $CD34^+$ cells as "human FLK^+ stem cells." Thus, the Examiner reasons that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to apply the teaching of Bruder to that of Lemischka to substitute isolated human mesechymal stem cells with isolated human KDR^+ stem cells to obtain the claimed method. The rationale for the motivation to make this combination, according to the Examiner, is "a recognition, expressly or implicitly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent that some advantage or expected beneficial result would have been produced by their combination." The Examiner cited Lemischka as teaching that isolated human FLK^+ stem cells have an ability to differentiate *in vitro* or *in vivo* and that this ability has important therapeutic applications. In response to Applicant's prior arguments, the Examiner pointed out that they did not adequately address the combination of the references.

Applicant again respectfully traverses the rejection.

The Examiner had made an obviousness rejection, because the references cannot support a rejection based on anticipation or lack of novelty. Thus the combination of references. The motivation to combine the references seems to originate from the quoted rationale of some innate "advantage or expected beneficial result [that] would have been produced by their combination."

Here, the combination appears to have been based on the hindsight provided by Applicant's own application, as the Examiner has specifically relied on two statements from the application. Such a combination, in the absence of any other motivating factor appears to be both unreasonable and inappropriate in supporting an obviousness rejection. Once an applicant discloses his or her invention the disclosure itself cannot reasonably be relied upon to support a combination of other references.

Here, one must objectively ask why a person skilled in the art, in the absence of the present disclosure, would combine Lemischka with Bruder. While they both relate to identification and characterization of stem cells and the desirability that the stem cells be used therapeutically if and when they differentiate into other tissues, they do not teach or suggest a method involving the isolation or use of KDR^+ stem cells. The objective analysis is especially important to keep from slipping, even inadvertently, into a hindsight obviousness analysis, which is not allowed.

Thus, when objectively analyzing the teaching of the references, it is important to understand what each teaches, and that each teaches something other than what Applicant is claiming in the present application. The combination of different teachings cannot result in Applicant's invention in the absence of the hindsight provided only by Applicant's own disclosure. Each reference approaches its issue differently from each other and from Applicant's method, as explained in detail in Applicant's response filed January 29, 2004, which will not be repeated here, but is incorporated by reference herein. One skilled in the art, having both references before him or her, among the myriad references that exist, would not come to a realization absent the spark provided only by Applicant that their distinct teaching should be combined in any way, let alone in a way that assertedly renders Applicant's invention obvious. To do so ignores the separate teachings of each reference. Only an artificial combination is possible and that is inappropriate for supporting an obviousness rejection.

Reconsideration and withdrawal of the obviousness rejection is respectfully requested.

Since all of the grounds for rejection have been overcome, Applicant respectfully solicits an early Notice of Allowance of all claims.

Respectfully submitted,

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Attachments:

Petition for Extension of Time
Request for Continued Examination
Articles:

R. Botta et al., The FASEB Journal, express article 10.1096/fj.03-0879fje, published online July 1, 2004
E. Pelosi et al., 2002, Blood, **100**, pp. 3203-3208
P. Madeddu, et al., The FASEB Journal, express article 10.1096/fj.04-2192fje, published online September 2, 2004